The Birds and the Bees and the Flowers and the Trees: Lessons from Genetic Mapping of Sex Determination in Plants and Animals

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Review

The Birds and the Bees and the Flowers and the Trees: Lessons from Genetic Mapping of Sex Determination in Plants and Animals

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ABSTRACT

The ability to identify genetic markers in nonmodel systems has allowed geneticists to construct linkage maps for a diversity of species, and the sex-determining locus is often among the first to be mapped. Sex determination is an important area of study in developmental and evolutionary biology, as well as ecology. Its importance for organisms might suggest that sex determination is highly conserved. However, genetic studies have shown that sex determination mechanisms, and the genes involved, are surprisingly labile. We review studies using genetic mapping and phylogenetic inferences, which can help reveal evolutionary pattern within this lability and potentially identify the changes that have occurred among different sex determination systems. We define some of the terminology, particularly where confusion arises in writing about such a diverse range of organisms, and highlight some major differences between plants and animals, and some important similarities. We stress the importance of studying taxa suitable for testing hypotheses, and the need for phylogenetic studies directed to taxa where the patterns of changes can be most reliably inferred, if the ultimate goal of testing hypotheses regarding the selective forces that have led to changes in such an essential trait is to become feasible.

THE ever-increasing accessibility of genetic markers is allowing sex-determining regions to be genetically mapped in a growing number of nonmodel organisms. There are several reasons for studying sex determination. In animals, gonadal differences are often accompanied by striking somatic secondary sexual dimorphisms, which are interesting in an evolutionary context (Shine 1989; Badyaev 2002). In plants, females and males often differ in flower morphology and abundance (Dawson and Geber 1998), and, although sex differences are often minor outside the flowers (or inflorescences), they do exist (Dawson and Geber 1998; Eppley and Wenk 2001). The genetic control of these phenotypes is a fundamental biological process, and studying sex determination pathways is important in animal developmental biology (Adams and McLaren 2002; Pinyopich *et al.* 2003), including genetic pathway evolution (Wilkins 1995; Williams and Carroll 2009).

Until recently, sex determination was generally studied by testing for genetic control *vs.* partial or complete environmental influences. Genetic systems were examined cytologically to determine the level of heteromorphism between the sex chromosomes and to identify whether females or males are heterogametic (see the comprehensive review in Bull 1983). Male heterogametic systems, referred to as XY, were also tested to identify whether the Y chromosome carries a male-determining gene, as in almost all therian mammals and most dioecious plants so far studied, or whether sex is determined through X–autosome balance, as in Drosophila and *Caenorhabditis elegans* (Haag 2005). For female heterogametic (ZW) species, analogous tests were used to identify how femaleness is determined.

Until recently, sex-determining genes and regions could be genetically mapped in only a few model species, but now that molecular genetic markers can be developed in nonmodel species, new information is becoming available about how genetic sex determination (GSD) mechanisms have changed during evolutionary history. It has long been known from genetic mapping in model systems, including mammals, and (more recently) birds, that sex chromosomes often have large nonrecombining regions (Bull 1983; Charlesworth 1991; Charlesworth *et al.* 2005). However, in other organisms, nonrecombining regions are not always large and may sometimes be absent. The evolution of sexual reproduction and recombination have been the focus

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of many years of discussion in evolutionary biology (Otto 2009), and studies of sex chromosomes are important for understanding why recombination is often lost, and elucidating the evolutionary consequences of recombination suppression (Charlesworth 1996; Otto and Barton 1997; Barton and Charlesworth 1998). The adaptation of genes on the sex chromosomes is also interesting, because this location affects the outcome of sex-specific selection pressures (Rice 1984; Charlesworth *et al.* 1987; Vicoso and Charlesworth 2006; Mank 2009a). Finally, the mechanism of sex determination can affect sex ratios (West and Sheldon 2002; Dorken and Pannell 2008; West 2009) and is therefore significant in evolutionary ecology.

Sex determination is also relevant in applied biology. In many domesticated animals, one sex may be of greatest economic interest to farmers and breeders. Modern meat production is largely based on males, including industrial production of chicken, cattle, and many fish, whereas females are the sex required for milk (cattle) and egg (chicken) production. Similarly, a few crop plants are dioecious, and, in some of these, the crop is produced by females (*e.g.*, grapes, dates, and papaya), while in other species the sexes differ in characteristics such as fiber or chemical content. Because immature birds, fish, and plants have no obvious phenotypic sex differences, maximizing agricultural returns often requires genetically sexing juveniles. Mapping sex determination is an important first step toward identifying the sex-determining genes or finding other sex-specific markers to develop molecular sexing methods.

In this review, we first summarize recent developments in genetic mapping of sex determination, concentrating on nonmodel plants and animals with genetic sex determination. We show how this information can be useful for understanding the evolution of sex determination and sex chromosomes and identify some important unanswered questions.

SOME IMPORTANT CONCEPTS AND TERMINOLOGY

As we will describe, genetic results have revealed changes in both the mechanism of sex determination and in the location of preexisting sex-determining genes. Given our wide taxonomic scope, it is important to note, first, that the terms X and Y chromosomes (or Z and W) do not necessarily mean a chromosome pair carrying the genes giving the earliest sex-determining signal to the developmental system. In some systems, the sex chromosomes and autosomes jointly determine sex, such as the X–autosome balance systems of Drosophila, *C. elegans*, and the plant Rumex (Navajas-Perez *et al.* 2005). Cases such as these probably evolved from ancestors whose sex chromosomes did carry such genes. Mechanisms of sex determination often vary among closely related taxa, and there are several hypotheses to explain transitions between different systems (*e.g.*, Bull 1983; van Doorn and Kirkpatrick 2007; Vuilleumier *et al.* 2007; Kozielska *et al.* 2010), but here we focus on new data about empirically demonstrated transitions, for which genetic mapping results are particularly illuminating.

Second, unlike the well-known sex chromosomes of therian mammals and birds, sex-determining chromosomes in some species lack large nonrecombining regions (Bull 1983). This situation can arise in two ways (Figure 1). Sometimes a sex-determining region has not yet evolved a nonrecombining region; this is often referred to as a proto sex chromosome. (See glossary of terms in Table 1.) Alternatively, a recent replacement of the sex-determining locus can produce a homomorphic chromosome pair with single-gene sex determination, and also no nonrecombining region. Replacement of an existing sex-determining gene by a new one will often lead to production of homozygotes for the original sex-determining locus, except when the change involves a fully dominant male-determining gene. When the original sex-determining locus lies within a large sex-specific nonrecombining region, such as a genetically degenerated Y chromosome that lacks many genes carried by the X, such YY individuals will often be homozygous lethal, preventing fixation of the new gene. However, such replacements could occur in plant and fish species where homozygotes for the sex-determining locus are viable. Such recently arisen sex-determining chromosomes are thus also called proto sex chromosomes. If a nonrecombining sex-determining region, such as a dominant male-determining region of a Y chromosome, moves to a new genomic location (*e.g.*, in a chromosome fusion), the original nonrecombining region may be retained. If not, the new sex-determining chromosome may evolve suppressed recombination, either immediately through the chromosome rearrangement itself, or through a later decrease in crossing over.

Given these varied possibilities, we use the term "sex-determining chromosome" for homomorphic situations, and reserve "sex chromosome" for situations when a large chromosome region is nonrecombining and there is a genetically degenerate Y or W chromosome. When reviewing plants, we follow convention and use the word "gender" to indicate maleness and femaleness of diploid individuals in species with separate sexes (dioecy) and also to haploid gametophytes of species where these produce only male or female gametes. For diploid plants, we also use the conventional terms "ovules and pollen" for the haploid stage. For gonochoristic animals, we use the word sex to refer to whether an individual is male or female, and refer to their gametes as sperm and eggs, respectively. There should be no confusion with the alternative meaning "sexual reproduction" (as opposed to asexual).

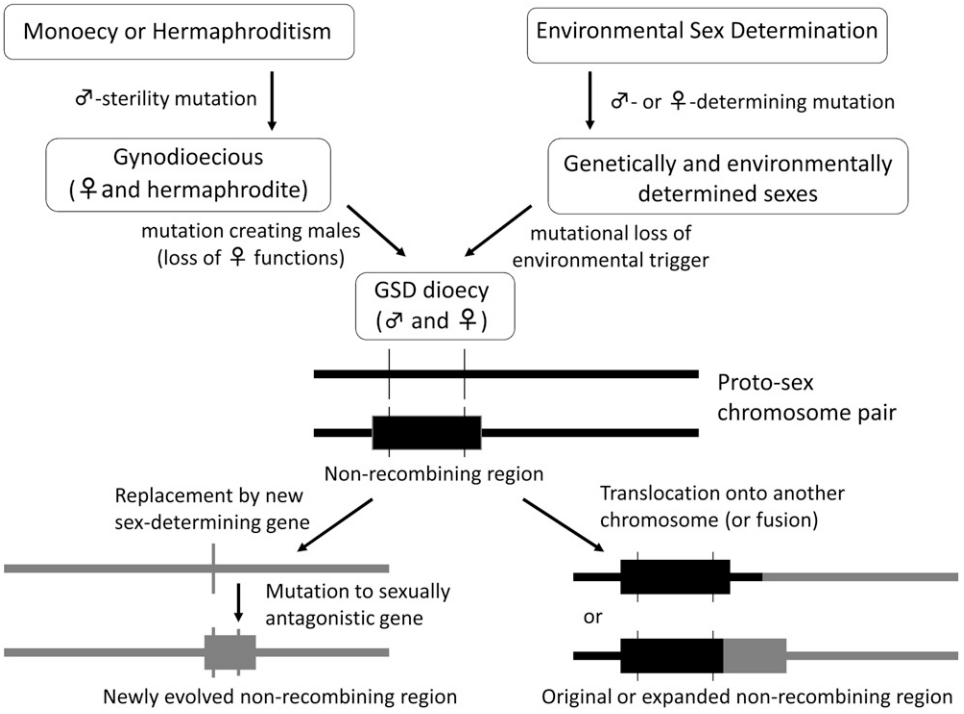

Figure 1.—Potential changes during the evolution of sex-determining regions from different possible starting states and possible changes in their subsequent evolution. In the evolution of dioecy from hermaphroditism, females are assumed to evolve first through a recessive male-sterility mutation. Starting from ESD, females might arise by loss of the ability to respond to an environmental trigger for male development. Males in either pathway then arise by a linked mutation causing failure to develop female structures, resulting in proto-sex chromosomes. Before recombination suppresssion has become extensive and genetic degeneration of one sex chromosome has occurred, the linkage group associated with sex determination can change by the replacement of a new sex-determining gene or via the transposition of the existing sex-determining region to a new autosome. Translocations between the sex chromosomes and autosomes may also occur, and may involve either proto-sex chromosomes or degenerated ones.

GENETIC MAPPING OF SEX-DETERMINING REGIONS

Table 2 summarizes plant and animal studies that have mapped the genomic region or regions associated with GSD. The term GSD is somewhat vague, and here we use it to mean that the alleles or alternative types of a particular linkage group (such as a Y or W chromosome) influence the probability of developing as a male or a female. Genetic control of sex need not be complete—in many organisms, environmental conditions, experimental treatments, or the genetic background, can affect the phenotypic sex of individuals. Our table therefore excludes species thought to have wholly environmental sex determination (ESD), but includes species where genetic influences have been demonstrated, even if environmental influences also affect or modify individuals' sex. The table also distinguishes between species with heteromorphic sex chromosomes (male or female heterogamety) and those where one sex is heterozygous for a sex-determining region, but without overt heteromorphism (in which case we specify whether females or males are the heterozygous sex).

In plants, "sex determination" is sometimes used to mean control of the floral organs involved in pollen and ovule production, as well as the developmental genetics of sterility mutants, such as gynoecious melons, without normal male functions (Martin *et al.* 2009). Here, however, we maintain the usage that prevails in animal genetics and use this term only for genes affecting whether the individual plant develops as a male or a female. We will therefore not consider cytoplasmic male sterility in plants (*e.g.*, Fishman and Willis 2006; Chase 2007). We also exclude fungal mating-type genes (Fraser *et al.* 2004), because these species are not dioecious and have no gamete dimorphism resembling sperm and eggs. For the same reason, we excluded mating-type regions of isogamous algae (Ferris *et al.* 2002).

Most of the mapping studies are based on linkage analysis of genetic markers typed in families, but FISH studies have also been very valuable, and genome sequencing is likely to become increasingly important. Most mapping so far has not identified the sex-determining genes (these are still known in only a few well-studied species), but has located the region(s) controlling sex determination (Holloway *et al.* 2000; Hawthorne 2001; Lee *et al.* 2004; Solignac *et al.* 2004; Telgmann-Rauber *et al.* 2007; Spigler *et al.* 2008).

Finally, recombination rates can be estimated separately in the male and the female parents of families (Hedrick 2007; Mank 2009b). In some species, recombination is absent, or nearly absent, throughout the genome in the heterogametic sex (Huxley 1928; Berset-Brändli *et al.* 2008). More often, recombination is suppressed in one sex in limited genomic regions (Naruse *et al.* 2000; Chen *et al.* 2007b; Yin *et al.* 2008), including the familiar situations in organisms such as mammals with no crossing over across most of the X and Y chromosomes in males. Recombination suppression in the region surrounding the sex-determining locus is a first step in the evolution of heteromorphic sex chromosomes (Figure 1).

TABLE 1

Glossary of terms

Term	Definition
Dioecy	plant species with separate male and female individuals (see gonochorism in animals, below).
Environmental sex determination (ESD)	sex-determining system where maleness or femaleness is induced by an environmental cue during development, such as the temperature control of sex in alligators and crocodiles.
Genetic sex determination (GSD)	the situation when alleles or alternative types of a particular linkage group (such as a Y or W chromosome) influence the probability of developing as a male or a female.
Genetic degeneration	the absence (or lack of function) of Y-linked alleles (in male-heterogametic systems), or W-linked ones (in female-heterogametic species) of many genes carried by the homologous sex chromosome.
Gonochorism	the term in animals referring to species with separate males and females (see dioecy in plants, above).
Hermaphroditism	in animals, species where individuals have both male and female gonads at the same time (simultaneous hermaphroditism), or an ovotestis, or where an individual may change sex (sequential hermaphroditism) during its life cycle. In plants, hermaphroditism denotes species whose flowers have both male and female parts.
Heterogamety	sex chromosome system in which males or females have heteromorphic sex chromosomes. In male heterogamety (including most mammals, Drosophila, and dioecious species of the plant Silene), males are XY and females XX, and in female heterogamety, including in birds and Lepidoptera, females are ZW, and males ZZ. Heterogamety is also sometimes used when the sex chromosomes are homomorphic (nonheteromorphic) to indicate which sex is heterozygous for the sex-determining region.
Monoecy	plant species with separate male and female flowers on the same individual plant.
Neo-sex chromosomes	systems where a sex chromosome has undergone a fusion or translocation with a formerly autosomal chromosome.
Proto-sex chromosomes	systems where a chromosome carries a newly arisen sex-determining gene or a newly evolved sex-determining region, but recombination suppression has not yet evolved and therefore there is no sex chromosome heteromorphism.
Sex chromosome heteromorphism	the degree to which the X and Y chromosomes (or Z and W chromosomes) differ from one another. This condition gives rise to either male or female heterogamety.
Subdioecy	presence of hermaphrodites as well as male and female individuals, found in some plant species populations.

Sex determination in plants and genetic mapping of sex-determining loci: We review data from plants first, because many dioecious plants probably evolved recently from hermaphrodite ancestors, and this recent origin is likely to underlie some major differences in the genetics of sex determination between plants *vs.* those animals with much older sex chromosomes, such as mammals and birds. In plants, as in animals, hermaphroditism or environmental sex determination (*e.g.*, Talamali *et al.* 2003) are possible ancestral states from which dioecy could have evolved. Dioecy in plants may also evolve from monoecy, where separate male and female flowers exist on the same individual (Renner and Ricklefs 1995; Dorken and Barrett 2004). In any of these ancestral states, all the genetic information required for the production of both anthers and pistils is present in each individual. The evolution of GSD must therefore involve mutations causing male and female sterility.

Dioecy in plants probably often evolved from hermaphroditism or monoecy by an initial recessive male-sterility mutation (which guaranteed outcrossing and resulted in a polymorphic population with females and hermaphrodites, see Figure 1), followed by one or more mutations increasing male fertility, creating males (or male-biased hermaphrodites) but reducing female functions (Charlesworth and Charlesworth 1978). When these later male-defining mutations occur in a linked region on the homologous chromosome to that containing the male-sterility allele, this creates a proto-Y chromosome (Westergaard 1958; Charlesworth and Charlesworth 1978). Because the initial male-sterility mutation is often recessive (homozygous individuals develop as females), the model predicts that females will be the homozygous sex and that the later female-suppressing mutations must be at least partially dominant. This model therefore predicts a predominance of male heterogamety in recently evolved dioecious species (Charlesworth and Charlesworth 1978), suggesting that female heterogamety may derive from male heterogametic ancestors.

Comprehensive reviews of dioecious plants indeed find that males are mostly the heterozygous sex (Westergaard 1958; Bull 1983). Among the best studied dioecious taxa, male heterogamety has evolved twice in the genus Silene (Mrackova *et al.* 2008), twice more in the

Hawaiian genus Schiedia in the same family, Caryophyllaceae (Sakai *et al.* 2006), and twice yet again in the genus Rumex in another family (Navajas-Perez *et al.* 2005). In all these cases, dioecy probably evolved within the past 10–15 million years (dioecy in Silene is estimated to have evolved more recently than in Rumex, and dioecious Schiedia evolved more recently still).

Female heterogamety has been demonstrated in the genus Cotula and other members of the daisy family (Lloyd 1975; Bull 1983), and in Populus (Yin *et al.* 2008). In these taxa, dioecy may be older (Populus is in the family Salicaceae, whose members are almost all dioecious, suggesting a considerable age for dioecy), and male heterogamety could exist in species not yet studied. In the subdioecious plant, *Fragaria virginiana* (with hermaphrodites as well as males and females) females are heterozygous. However, males and females appear to have evolved very recently; hermaphrodites are also present, and recombinants occur between the sex-determining genes (Spigler *et al.* 2008). This may be an unexpected instance of *de novo* evolution of female heterogamety, and fully dioecious Fragaria species, if they exist, may have evolved recombination suppression in the sex-determining region.

In several plants with male-heterozygous systems, suppressed recombination is limited to small regions of the sex-determining chromosomes; sometimes, no markers fully linked to the sex-determining region have been discovered, even with dense mapping (Table 2). Possibly these species evolved dioecy recently, and there has simply not been enough time to evolve suppressed recombination across the entire sex-determining chromosome pair. Alternatively, evolution of suppressed recombination across most of the chromosome pair may not be inevitable (see our later discussion of the evolution of recombination suppression below).

The evolution of dioecy has been dated in only a handful of plant taxa, leaving it currently unclear whether all plant sex-determining chromosomes without large nonrecombining regions are young, and systems with heteromorphism older; there are no current mapping results for old angiosperm sex chromosomes. Phylogenetic studies suggest that dioecy evolved in the common ancestor of the families Begoniaceae, Cucurbitaceae, Datiscaceae, and Tetrameleaceae, probably from hermaphroditism (Zhang *et al.* 2006), and it has probably been lost many times in the first two large families (Mabberley 1997). This suggests considerable age, consistent with known XY heteromorphic sex chromosomes in dioecious Cucurbitaceae (Sinha *et al.* 2007). By mapping genes to these chromosomes, it may be possible to estimate the chromosomes' age using sequence divergence between X–Y ortholog pairs and to determine whether the same genomic region is involved in sex determination in all these taxa.

The ages of more plant sex chromosomes should be estimated, including those of species with undifferentiated potentially proto-X–Y pairs, such as papaya and the moss *Ceratodon purpureus* (see Table 2). It will therefore be important to sequence genes in the nonrecombining regions. In papaya, many sex-linked nongenic markers (mostly AFLPs) were found in a physically small nonrecombining sex chromosome region (Liu *et al.* 2004), which suggests high divergence of the male-specific region. However, the four papaya X–Y gene pairs so far studied all have synonymous site divergence <10%, suggesting that recombination stopped quite recently in this part of the chromosome pair (Yu *et al.* 2008). Genes from other locations in the papaya nonrecombining region are, however, needed to test whether the entire male-specific region evolved suppressed recombination recently.

In many plants, the genetic basis remains unclear, including stinging nettles *Urtica dioica* (Glawe and Jong 2009), *Byronia dioica* (Correns 1928), and *Antennaria dioica* (von Ubisch 1936), but males are again probably the heterozygous sex and females homozygous (reviewed in Westergaard 1958). These cases may involve recently evolved dioecy, breakdown of dioecy, or plants whose gender is subject to environmental influences. More phylogenetic and genetic studies (including using genetic markers) should clarify these situations.

Despite their fairly recent origins, some modifications of XY systems have occurred in several plants. XY_1Y_2 neo-sex chromosome systems have arisen in *Humulus japonicus* (Kihara and Hirayoshi 1932; Kim *et al.* 2008), and *Silene diclinis* (Howell *et al.* 2009), and twice independently in Rumex (Smith 1963, 1969; Navajas-Perez *et al.* 2005), and *Viscum fischeri* has a multiple Y system (Barlow and Wiens 1976). These situations probably evolved by X–autosome translocations (Figure 1; changing the ancestrally autosomal element into a chromosome that segregates from the new arm of the X, denoted by Y_2, while Y_1 denotes the ancestral Y), although fission of the Y cannot yet be ruled out in Rumex; genetic maps using genic markers might be able to establish whether the Y chromosomes still carry functional genes (as in Silene; see for instance Marais *et al.* 2008), in which case their gene content could be compared and this possibility tested.

Within the genus Rumex, an X–autosome balance sex-determining mechanism has also evolved, apparently from a male-determining Y mechanism retained in most species (Navajas-Perez *et al.* 2005). It is less clear whether the few known plants with female heterogamety (ZW systems) evolved from XY systems, *vs. de novo* evolution of female heterogamety (see above). It will be interesting to discover whether taxa with known ZW dioecious plants, have relatives with male heterogamety. If so, phylogenetic studies like those in Silene (Desfeux *et al.* 1996) and Rumex (Navajas-Perez *et al.* 2005) might reveal transitions between XY and ZW sex chromosomal systems.

Overall, the results from plants indicate that dioecy has evolved many times, sometimes quite recently,

through major loss-of-function mutations producing unisexual females and males, usually with a male-determining "Y" chromosome. Plant sex-determining regions are probably often small, consistent with viability of homozygotes for the Y chromosome in some species (Westergaard 1958). However, few species have been genetically mapped, and the physical sizes of the regions are mostly unknown, because finding multiple sex-linked markers can imply either a large physical size or a large divergence time. More phylogenetic studies are needed to determine ancestral *vs.* derived states and to estimate the ages of more sex-determining chromosomes.

Genetic mapping of sex-determining loci in animals: Sex chromosome evolution in animals has mainly been studied using therian mammals (Potrzebowski *et al.* 2008), Drosophila (Koerich *et al.* 2008), and birds (Mank and Ellegren 2007). Within each of these taxa, GSD arose just once, long ago (Matsubara *et al.* 2006; Veyrunes *et al.* 2008). Despite originating separately, recent linkage maps of the therian mammal X and bird Z chromosomes have revealed that recombination between the sex chromosomes was initially suppressed over a limited region of the chromosome pair, and later, in both lineages, extended across most of the recombining "pseudoautosomal" genome regions, resulting in "evolutionary strata" (Lahn and Page 1999; Skaletsky *et al.* 2003; Ross *et al.* 2005; Nam and Ellegren 2008; see Figure 2). Similar strata are found in the plant *S. latifolia* (Bergero *et al.* 2007). In birds, heteromorphism between the Z and W varies greatly (Mank and Ellegren 2007); the few ratite birds so far studied have only slight heteromorphism, implying a small region of restricted recombination (Tsuda *et al.* 2007), whereas, in more recently derived bird lineages (Neognathae—all modern birds except ratites and tinamous), the W chromosome is much smaller than the Z (Solari 1993). *In situ* hybridization studies show that the nonrecombining part of the W chromosome in Neognathae has lost most genes that were present on the ancestral autosome (Nishida-Umehara *et al.* 2007; Nanda *et al.* 2008). Similar results have been found in snakes (Matsubara *et al.* 2006).

Compared with other animal clades, the sex chromosomes of mammals and birds are highly conserved, apart from addition of some repeated copies of testis-expressed genes to the Z and X chromosomes (Mueller *et al.* 2008; Ellott *et al.* 2010). Most therian mammals have XY GSD, and the X has changed very little since the addition, ~166 million years ago, of the *q* arm of the current placental X to the ancestral therian X, which remains the marsupial X (Waters *et al.* 2001). The gene content and gene order of the therian X chromosome have changed little (Murphy *et al.* 1999; Raudsepp *et al.* 2004; Delgado *et al.* 2009), apart from duplications of genes onto the X and movements of some genes off the X (Potrzebowski *et al.* 2009), and formation of neo-sex chromosomes in some species (reviewed in Bakloushinskaya 2009). The avian Z chromosome gene content and order are even more conserved (Dawson *et al.* 2007; Backström *et al.* 2008). As the existence of strata of different divergence levels between the sex chromosomes is the main new addition to our knowledge about the evolution of these chromosomes in mammals and birds to have emerged from genetic maps, and vertebrate sex determination was recently reviewed by Graves and Peichel (2010), the remainder of this review concentrates on recent genetic results on the genetic control of sex in nonmodel invertebrates and from fish, amphibians, and reptiles.

Invertebrates: GSD is found in many invertebrate taxa, and male heterogamety is widespread, although female heterogamety is documented in schistosomes (Platyhelminthes) (Taguchi *et al.* 2007; Criscione *et al.* 2009), the lepidoptera (Abe *et al.* 1998), and some crustaceans

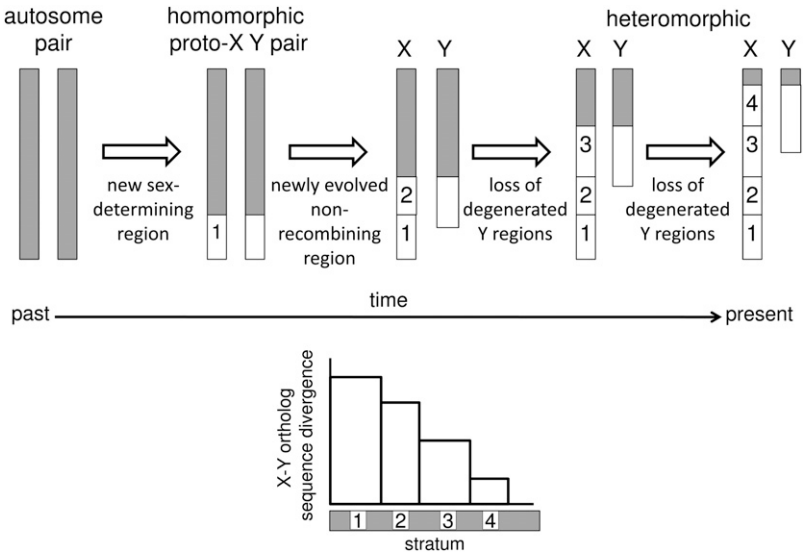

Figure 2.—Evolutionary strata are regions where recombination ceased at different times during sex chromosome evolution, as recombination suppression (open bars) spreads gradually over the length of the sex chromosomes. Regions where recombination ceased first have greater sequence divergence between X–Y or Z–W orthologous pairs of genes than regions where recombination ceased recently. In this illustration of an X–Y pair, sex-determining genes evolved in the initially recombining region labeled 1, and recombination ceased first in this region, creating stratum 1. This led to genetic degeneration of this stratum in the Y chromosome, and the evolution of chromosome heteromorphism. The process was repeated when recombination ceased in stratum 2, then stratum 3. The cessation of recombination in stratum 4 leaves only a small "pseudoautosomal region" on the X and Y chromosomes where recombination still occurs.

(Li *et al.* 2003; Zhang *et al.* 2007). Many species have X0 systems, including some nematodes and insects, and these most likely evolved from XY GSD (Shearman 2002), after genetic degeneration of the Y chromosome and movement of genes essential for male functions to other chromosomes. Such gene movement has been documented in *Drosophila pseudoobscura* (Carvalho and Clark 2005; Koerich *et al.* 2008; Larracuente *et al.* 2010). X0 species, such as *C. elegans* (Gladden *et al.* 2007), must also have a dosage-sensitive sex determination system, where the different amounts of one or more X chromosome transcripts between the sexes provide a "counting mechanism" relative to autosomal elements. It will be interesting to identify sex-determining mechanisms in other nematodes, and study their sex chromosomes, which may have undergone neo-sex chromosome formation and other changes (Streit 2008).

In insects, chromosome fusions and translocations, involving both the X (McAllister and Charlesworth 1999) and the Y (MacKnight 1939; Bachtrog and Charlesworth 2002) have occurred many times, creating nonrecombining neo-sex chromosome systems in species such as Drosophila, where recombination does not occur in the heterogametic sex. Neo-sex chromosomes have also evolved by fusions in X0 systems of grasshoppers (White 1973). Unlike Drosophila, many such species have crossing over in both sexes, and the added arm can continue to recombine, though rearrangements probably often decrease the chiasma frequency (reviewed in Marti and Bidau 1995; Charlesworth and Wall 1998). Some termite species have multiple translocations involving the sex chromosomes, with chiasmata largely in terminal regions (Luycx 1981). It is unclear whether such rearrangements were selected because it is advantageous to move genes to the sex chromosomes, and estimates of the relative rates of such events for both sex chromosomes and autosomes have not yet established whether the sex chromosomes are disproportionately involved in translocations (Charlesworth *et al.* 1987).

The prevalence of male-heterogametic GSD suggests that it was probably the ancestral state when separate sexes were first established in these insects. However, the long evolutionary time since the insect ancestor makes inferring the ancestral state difficult. Even when two taxa both have XY systems, they may not have evolved from a single ancestral XY system—a new GSD system could have re-evolved in a lineage after being lost. For instance, the mosquito *Anopheles gambiae* resembles Drosophila in having a large region in which the X and Y do not recombine. However, these species diverged ~250 MYA, and genome sequencing shows that the ancestral Drosophila X (the chromosome arm homologous to the *D. melanogaster* X, or the pre-fusion portion of the *D. pseudoobscura* X), carries few genes present on the X chromosome of *A. gambiae*, and, conversely, most *A. gambiae* X chromosome genes are scattered on various Drosophila autosomes (Zdobnov *et al.* 2002). Even among mosquitoes, the sex-determining genes may have been replaced. Aedes mosquitoes (like other Culicinae) have no sex chromosome heteromorphism, and so far no nonrecombining region has been found in the *Culex tarsalis* sex-determining chromosome (Presgraves and Orr 1998; Venkatesan *et al.* 2009). However, one arm of the *A. aegypti* sex-determining chromosome is probably homologous with the *D. melanogaster* X (Nene *et al.* 2007).

In several Dipteran insects, sex-determining genes are known to move their chromosomal locations without major chromosomal rearrangements. Some housefly populations have an XY system, and it is unknown whether the different genomic locations of sex-determining regions in other populations represent gene movements or replacements by new sex-determining genes (reviewed in Hediger *et al.* 2010). In *Megaselia scalaris*, there are no heteromorphic sex chromosomes (Traut *et al.* 1999), and a male-determining factor may have moved around the genome within a transposable element (Traut and Willhoeft 1990). This species may allow tests of whether a nonrecombining sex chromosome-like region has evolved from a single-gene system, but it is currently unclear whether there is indeed an associated nonrecombining region (Traut and Wollert 1998). Information about the existence of a nonrecombining region should emerge from new approaches for obtaining genetic markers and from genome sequencing (Rasmussen and Noor 2009).

Although some of these changes could be due to movements of existing genes, sex-determining systems and genes differ among insects. Replacement of a sex-determining gene by another gene, on the same or a different chromosome, may be involved in the transitions between XY and ZW systems (for example, in the hessian fly, *Mayetiola destructor*, a new sex-determining gene competing with the ancestral system of male heterogamety seems to be present on a neo-W chromosome; Benatti *et al.* 2009), or from a male-determining Y to an X–autosome balance or dosage-sensitive system. If sex-determining genes can be identified, it should become possible to distinguish such new sex-determining genes from movements of existing sex-determining genes.

Replacement of the sex-determining genes by a new gene controlling maleness or femaleness in insects often leaves the downstream parts of the pathway largely unchanged (Wilkins 1995; Graham *et al.* 2003; Pane *et al.* 2005; Siegal and Baker 2005; Cho *et al.* 2007). An example is the major change in the mechanism of sex determination represented by the haplodiploid system in Hymenopteran insects (reviewed by Bull 1983), where females lay either fertilized eggs that generally develop as females, or unfertilized ones that develop as haploid males.

Even among different Hymenoptera, the systems differ. In the wasp *Nasonia vitripennis*, sex is determined

through imprinting of the *N. vitripennis transformer (Nvtra)* locus (transformer is a gene already known to act in the Drosophila sex-determining pathway; see Marin and Baker 1998). The maternal *Nvtra* copy is silenced in developing embryos, and without transcription from this locus, individuals develop as male. Unfertilized eggs (with only a maternal *Nvtra* copy) therefore develop as males, while fertilized eggs (expressing the paternal copy) develop as females (Verhulst *et al.* 2010). In bees, however, the signal to switch the developmental system to male or female involves "complementary sex determination" (csd), with a polymorphic locus, such that most diploid zygotes are heterozygous, and possession of different alleles serves as a signal that controls female development, while haploid eggs (or homozygous ones produced by inbred pairings) develop as males.

Even among different bees, one sex-determining gene has been replaced by another; in different species, the functional csd gene differs (confusingly, since the notation *csd* is used for the different genes). The gene with the csd function in honeybees (*Apis mellifera*) was found to be a duplicated and changed form of a preexisting gene, *fem*. The *fem* gene is present in many non-Apis Hymenopteran species, including a bumblebee (Bombus), a stingless bee, Melipona, and even *N. vitripennis*, and is involved in a downstream step of sex determination, but not in the csd function (Hasselmann *et al.* 2008a). *fem* apparently controls the female–male switch through expression of alternative RNA transcripts in a system reminiscent of Drosophila sex determination (see Marin and Baker 1998; Siegal and Baker 2005). However, in honeybees and their close relatives *A. cerana* and *A. dorsata*, which diverged in the last 10 million years, *fem* is duplicated. The duplicate gene has taken over the *csd* function from an unknown gene that presumably had this function in an ancestor, while *fem* maintains the female developmental fate (Hasselmann *et al.* 2008a,b; Gempe *et al.* 2009).

Fish: The fish ancestor is thought to have had separate males and females (Smith 1975), as simultaneous hermaphroditism is rare in the clade (Devlin and Nagahama 2002) and confined to the tips of the fish phylogeny, suggesting recent origins (Mank *et al.* 2006). However, determining whether the fish ancestor had GSD or ESD is difficult, because sex-determining mechanisms are known in only a small fraction of fish, and sex determination has undergone many changes (Figure 3 and reviewed in Volff *et al.* 2007), and the phylogeny of many higher-level fish taxa is unresolved (Mank *et al.* 2006; Setiamarga *et al.* 2009). In some clades, dioecy may have evolved from sequential hermaphroditism, which is widespread in fish, including the closest relatives of the androdioecious goby, *Lythrypnus dalli* (Drilling and Grober 2005), which could be in transition to or from dioecy.

Thus, although fish are not ideal organisms for studying the initial evolution of separate sexes, several taxa are excellent for studying modifications of GSD (Mank *et al.* 2006), and some may allow studies of the evolution of GSD from initial states other than simultaneous hermaphroditism. A particularly interesting situation is when there is single-gene control after a change in the sex-determining system. Although sex chromosome heteromorphism is known in some fish (see Table 2), many species have homomorphic sex-determining chromosomes and could have single sex-determining genes (and should, strictly, not be called XY or ZW systems). It is currently unclear whether such new sex-determining genes can trigger the evolution of a nonrecombining sex chromosome-like region, representing a different origin for sex chromosomes from that in plants. If cases of young and heteromorphic sex chromosomes are found, they will be ideal for testing what has driven recombination suppression.

Many fish have large clutch sizes that facilitate linkage mapping, and genetic mapping in fish has revealed several new cases of GSD. For instance, genetic mapping in catfish (order Siluriformes) found a sex-determining chromosome with a small nonrecombining region, which is heterozygous in males (Waldbieser *et al.* 2001). In the hirame, a flounder (order Pleuronectiformes), males are heterozygous (Coimbra *et al.* 2003), but temperature also influences sex development (Yamamoto 1999). Another pleuronectiform, the half-smooth tongued sole, has female heterogamety, and many genes present only in females have been identified through a BAC library screen, suggesting W linkage (Shao *et al.* 2010). Among fish whose genomes have been sequenced, the pufferfish (*Takifugu rubripes*, order Tetraodontiformes) has a male-heterozygous region (Kikuchi *et al.* 2007). In the zebrafish (*Danio rerio*, Cypriniformes), sex is determined by a combination of genetic and environmental influences (Siegfried and Nusslein-Volhard 2008), while among its relatives, the goldfish has an XY system of GSD (Zhang and Wu 1985) and Leporinus (Characiformes) has a strongly heteromorphic ZW system, with largely heterochromatic W chromosomes, which have probably undergone W–autosome translocations (Parise-Maltempi *et al.* 2007).

Many of these findings are from distantly related isolated species, among which it is impossible to recognize large syntenic blocks or, until the sex-determining loci are identified, determine gene homology. Comparative mapping in fish is further complicated by the fact that several whole genome duplication events have occurred within the clade (Meyer and Schartl 1999; Christoffels *et al.* 2004; Mank and Avise 2006). However, five clades have provided information about changes in sex-determining loci and could potentially lead to estimates of turnover rates.

In sticklebacks, genetic mapping has been important for evolutionary studies of adaptation and has led to the

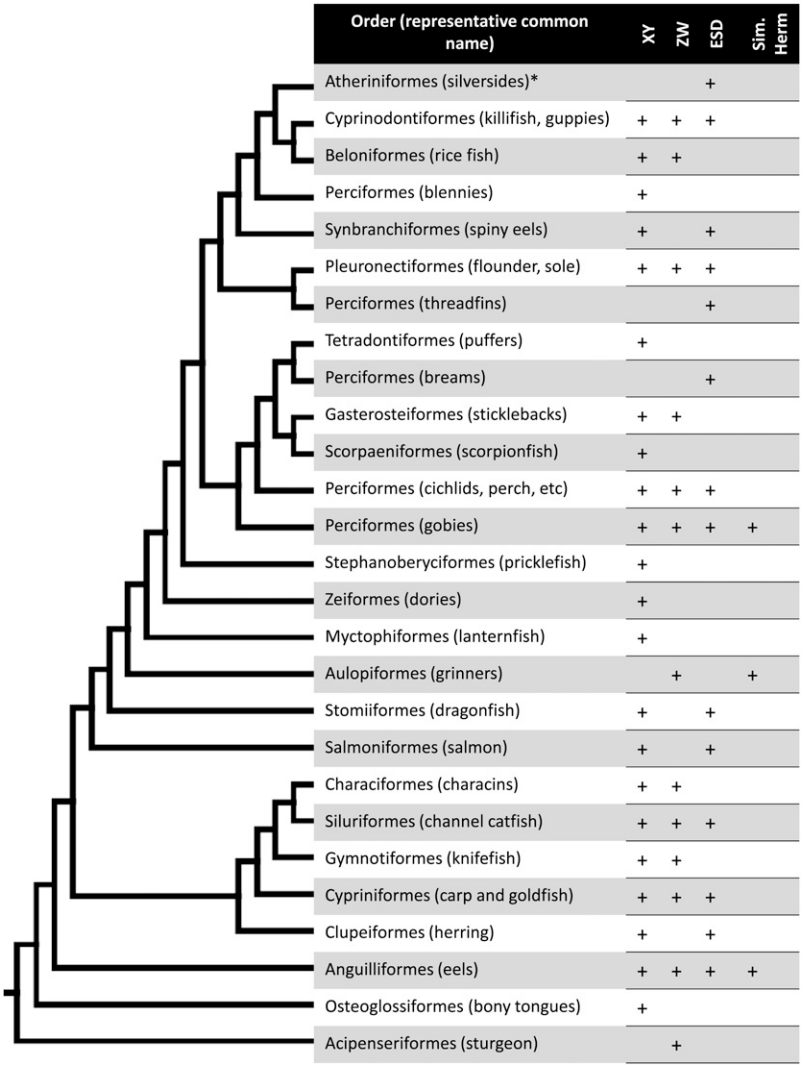

FIGURE 3.—Sex determination in the actinopterygiian fishes (adapted from MANK *et al.* 2005, 2006). Shown is the supertree topology for the clade, presenting only the orders where sex determination is known for at least some members. Divergence dates of teleost clades are still contested and are therefore not indicated in the figure; however, it is helpful to note several nodes dated with a recently estimated molecular clock (SETIAMARGA *et al.* 2009). These include the origins of the Tetraodontiformes (80 MYA), the Beloniformes (115 MYA), the Salmoniformes (135 MYA), the Cypriniformes (190 MYA), and the Anguilliformes (220 MYA) and the divergence of the Gasterosteiformes from the Scorpaeniformes (150 MYA). Under environmental sex determination (ESD), we include sequential hermaphrodites where size plays a role in determining sex, as well as gonochorist species, where the environment can influence sex determination during development, and taxa with sequential hermaphrodites are noted separately (sim. herm.). Male heterogametic species are denoted by XY, even when there is no known chromosome heteromorphism, and female heterogametic species are similarly denoted by ZW. Sex determination in silversides has been shown to have a genetic component, although it is not known whether males or females are the heterozygous sex (CONOVER and KYNARD 1981).

development of a panel of molecular genetic markers (PEICHEL *et al.* 2001; SHAPIRO *et al.* 2009). Their use in different species in the family Gasterosteidae has revealed male heterogametic GSD in both *Gasterosteus aculeatus* and *Pungitius pungitius*. Both have sex chromosome heteromorphism, suggesting a large nonrecombining region (ROSS and PEICHEL 2008; SHAPIRO *et al.* 2009). The X_1X_2Y GSD system in *G. wheatlandi* (the sister species to *G. aculeatus*) is probably derived by a Y–autosome fusion involving an XY pair in a common ancestor (ROSS *et al.* 2009); surprisingly, this fused the linkage groups corresponding to the X chromosomes in *G. aculeatus* (LG19) and the more distant relative, *P. pungitius* (LG12). It is uncertain whether the ancestor of *G. aculeatus* and *P. pungitius* had an XY system (implying replacement or movement of the male-determining gene in one species) or whether one of these lineages evolved this independently from a different ancestral state. A more distant relative, *Apeltes quadracus*, has female heterogamety with visible chromosome heteromorphism. In *Culaea inconstans* (sister to *P. pungitius*), there is no evidence of heteromorphic sex chromosomes or GSD, although markers linked to a sex-determining gene may be found once more markers and chromosomes are mapped in this species (ROSS *et al.* 2009).

The salmon family are somewhat similar. Early cytological studies showed clearly differentiated male heterogametic sex chromosomes in several species (THORGAARD 1977), and later genetic mapping in Oncorhynchus, Salvelinus, and Salmo confirmed male heterozygous GSD in most species, though with only slightly differentiated sex chromosomes and some environmental influence (NICHOLS *et al.* 2003; WORAM *et al.* 2004; GHARBI *et al.* 2006; ALFAQIH *et al.* 2008). However, like the sticklebacks, the sex-determining chromosomes in different species do not have the same gene content and probably did not evolve from a common single ancestral XY pair. Few genes are currently mapped, so the chromosomal homologies in the different species, and the evolutionary history of the changes, are not yet certain. Genetic mapping with

microsatellite loci is starting to indicate some chromosomal homologies, even between distantly related fish (Rexroad *et al.* 2008). However, these homologies have not yet been shown to extend to entire chromosomes having the same gene content in distant relatives, and fish chromosomes appear to have been extensively rearranged (Naruse *et al.* 2004) and it is unlikely that entire arms will be recognizable as they are in Drosophila and Hymenoptera. The X and Y chromosomes may have evolved *de novo* four or more times from different autosomes within the Salmonid family, or a sex-determining region may have moved to an autosome several times, creating a new, homomorphic sex-determining chromosome. It is also possible that a new sex-determining gene may have replaced a preexisting one in some species, and heteromorphism evolved later in some cases. A final alternative is that the recent whole genome duplication in this family created duplicate sex-determining genes, and different copies were lost in different lineages. Finer mapping could reveal syntenies and exclude or confirm these scenarios.

The medaka, *Oryzias latipes* (Cyprinodontiformes) is a clear case of a new sex-determining gene replacing an unknown preexisting one. Linkage mapping (Naruse *et al.* 2000) identified a sex-determining chromosome pair (9) containing a small (258 kb) region specific to males (Kondo *et al.* 2006). In this region, males are heterozygous, and there is no homology (and no recombination) with the homologous "X" chromosome (Kondo *et al.* 2006). Further genetic studies identified a duplicate of the DMRT1 gene in this region (Matsuda *et al.* 2002; Nanda *et al.* 2002). This duplicate "DMY" gene has superseded an ancestral gene previously controlling the maleness development pathway, which is presumably still involved in some congeneric rice fishes (Matsuda *et al.* 2002). In the region with homology between the X and Y that surrounds the sex-determining locus, recombination in males is lower than in females. Interestingly, when heterozygotes for the male-specific region are given a sex reversal treatment, making them female, they recombine at a rate like that of "XX" females (Kondo *et al.* 2001). The reduced recombination in males is therefore not directly caused by the insertion, but rather, depends on the sex phenotype. Sex-specific recombination rates have not been published for other genome regions, so it is unclear whether there are genome-wide differences in recombination.

Although DMRT1 homologs are involved in male determination in many animals, including Drosophila, *C. elegans*, mammals (Raymond *et al.* 2000; Haag 2005), and birds (Smith *et al.* 1999, 2009), the DMY gene is missing in several Oryzias species (Kondo *et al.* 2004; Tanaka *et al.* 2007), although these species retain the ancestral *DMRT1* locus. Additional linkage mapping indicates independent changes to female heterogametic GSD in *O. javanicus* and *O. hubbsi* (Takehana *et al.* 2008).

A particularly interesting group of fish for mapping studies includes the guppy, *Poecilia reticulata* and Xiphophorus, in the same superorder, Atherinomorpha, as medaka (Figure 3). Classical genetic work in both species found an XY GSD system and demonstrated that many male color patterns are caused by polymorphisms in genes fully or partially Y linked (Winge 1927; Kallman 1970). However, the platyfish, *Xiphophorus maculatus*, also has a "W" chromosome carrying a female-determining gene. Xiphophorus species may be changing between female and male heterogamety, and in *X. maculatus*, like the frog *Rana ragosa* (see below), the X and W chromosomes are at least partially homologous (reviewed in Schultheis *et al.* 2009). The male color polymorphisms may be maintained by sexual antagonism, being advantageous to males, but disadvantageous to females (Fisher 1931; Kallman 1970; Rice 1984). These species therefore hold promise for testing the hypothesis that sexually antagonistic genes have driven selection for reduced X–Y recombination (see Figure 1 and below). Large numbers of SNP markers have been obtained for genetic mapping in guppies, using EST sequences (Tripathi *et al.* 2009a,b), and new progress can now occur in comparing the maps of these species' sex chromosomes (Morizot *et al.* 1991, 2001). No marker completely linked to the sex-determining locus has yet been found in either species, but part of the guppy sex chromosome may recombine less frequently in males than females (Tripathi *et al.* 2009a). The guppy sex chromosome is homologous with the *O. luzonensis* sex chromosome (Tanaka *et al.* 2007) and with medaka autosome 12 (Tripathi *et al.* 2009b), raising the possibility that the guppy can help to identify the sex-determining region in the ancestor of the medaka. Recent estimates of the age of these taxa are large, suggesting that changes in sex determination may not be rapid.

In tilapia and cichlid species (family Cichlidae, in the ancient and polyphyletic order Perciformes), there are again species with sex-determining (nonheteromorphic) chromosomes called X, Y, Z, and W, plus environmental influences complicating genetic analyses (Baroiller *et al.* 2009; Ser *et al.* 2010). Tilapia are farmed for food, and males have higher growth rates than females in commercial production (Beardsmore *et al.* 2001), therefore a method to sex fingerlings would be valuable. However, changes in the sex-determining region may frustrate efforts to develop a single method to sex multiple species unless the same gene turns out to be involved across the clade.

Within the tilapiine cichlids (genus Oreochromis), use of genetic markers has shown that females are heterogametic in *O. karongae* (Cnaani *et al.* 2008) and *O. tanganicae* (Cnaani and Kocher 2008), while in *O. niloticus* males are the heterozygous sex (Cnaani *et al.*

2008), and *O. aureus* has a polygenic sex-determination system (Lee *et al.* 2004). In the closely related genus Tilapia, one species, *T. zillii*, has GSD with heterozygous males, and the sex-determining chromosome is homologous to that in *O. niloticus*. In *T. mariae*, females are heterogametic, and the sex-determining chromosome is the same as that in *O. karongae* (LG3), but different from that in the male heterozygous species, LG1 (Cnaani *et al.* 2008). The genetic maps of Lake Malawi cichlids are almost perfectly colinear with those of tilapia cichlids (Ser *et al.* 2010). In Lake Malawi cichlids, female heterogamety may have evolved recently from male heterozygosity (Roberts *et al.* 2009; Ser *et al.* 2010), and a similar transition may have occurred independently in Oreochromis (Cnaani *et al.* 2008). It will be necessary to study more sets of closely related species to infer the rates and directions of changes and to determine whether these changes are due to transpositions of sex-determining genes or to the origin of new sex-determining genes, coopting the sex-determination pathway.

Amphibians and reptiles: Most amphibians exhibit GSD and possess homomorphic sex-determining chromosomes (Eggert 2004). There have been many shifts between male and female heterogamety (Hillis and Green 1990), including among different populations of the Japanese frog, *R. ragosa* (Miura *et al.* 1998), where genetic mapping has shown that the X and W chromosomes are homologous (Uno *et al.* 2008). It is not yet understood how the change from one state to the other occurred, but the male heterozygous (XY) state is probably ancestral (Ogata *et al.* 2008). Genetic mapping in salamanders (Smith and Voss 2009) found that females are the heterozygous sex, and the sex-determining locus is not in a chromosome region with reduced recombination. In the European tree frog, *Hyla arborea*, males (the heterozygous sex) are nearly achiasmate (Berset-Brändli *et al.* 2008). The current genetic map is based on noncoding DNA so it is not yet known whether the male-specific region has degenerated or experienced gene loss.

In reptiles, temperature-based ESD predominates and was probably the ancestral state from which ZW GSD has repeatedly evolved, with few reversals (Janzen and Phillips 2006; Organ and Janes 2008). These inferences remain tentative, as there is no definitive reptile phylogeny (Olmo 2005; Pokorna and Kratochvil 2009). In the Australian dragon lizards, FISH mapping combined with phylogenetic analysis has demonstrated that female heterogametic sex determination evolved at least twice independently from temperature-sensitive ESD (Ezaz *et al.* 2005, 2009). Separate sexes and GSD in birds probably evolved independently of the change to GSD in reptiles, since the reptile DMRT1 homolog is autosomal (Kawai *et al.* 2007).

In animals, the transition from ESD to GSD may be more common than the reverse, although changes from GSD to ESD may have occurred in nematodes (Haag 2005). It has been suggested that ESD may be an "evolutionary trap" (Janzen and Phillips 2006; Pokorna and Kratochvil 2009) or that GSD may accelerate speciation, leading to most lineages having GSD, even with similar rates of transition (Organ *et al.* 2009). However, these alternative hypotheses are difficult to differentiate from each other, and no clear mechanism is known either to prevent reversion to ESD or promote speciation.

LESSONS FROM GENETIC MAPPING OF SEX-DETERMINING LOCI

Male and female heterogamety: In clades with multiple types of GSD, including reptiles, fish, and plants, males are generally more often heterogametic or heterozygous for the sex-determining region than females (Westergaard 1958; Janzen and Phillips 2006; Mank *et al.* 2006). However, as explained above, in animals it is often unclear whether sex determination evolved from a situation without GSD, or by movement of a preexisting sex-determining gene, or by creation of a new one. The relative frequencies of male and female heterogamety will depend on the rates of such changes.

It has been argued that male heterogamety may evolve more readily, because, in animals, femaleness is probably often the developmentally "default state," and maleness requires a positively male-determining gene (Raymond *et al.* 2000). With male heterogamety, the male-limitation of Y chromosomes (or Y-like regions) allows the male-determining gene to be restricted to males. Thus, female heterogametic GSD, where no genomic region has male-limited inheritance, may make it more difficult to specify male development. Known mechanisms of female heterogametic GSD indeed appear to be complex, with dosage-sensitive genes involved in male sex determination located on the Z chromosome of birds (Smith *et al.* 2009) and the lizard *Pogona vitticeps* (Quinn *et al.* 2007), and even more complicated interactions of the Z and W chromosomes in Xenopus frogs (Yoshimoto *et al.* 2008). However, femaleness is not always the default: in zebrafish, the germ line is required for ovary development, and in its absence the gonad develops as a testis (Siegfried and Nusslein-Volhard 2008).

Moreover, this argument can apply only to species whose GSD has evolved from a species that already has sex chromosomes, not to the initial stages of the evolution of separate sexes. Table 3 summarizes the types of selection that might be expected following the evolution of sex-determining regions from different ancestral states. In the initial evolutionary stage, when GSD evolved from hermaphroditism or ESD, the genes involved would not be confined to one sex. When GSD evolves from hermaphroditism in animals or monoecy in plants (diagrammed in Figure 1), there is, of course, no

default sex. If females evolve first (see above), this predicts that males will generally be the heterozygous sex, because loss-of-function mutations creating females are probably generally recessive, and females would be homozygous.

However, GSD in animals seems often to have evolved from ESD, rather than from a simultaneously hermaphroditic state. In species with ESD, femaleness could, of course, be the default state, with an environmental trigger inducing maleness. This could change to GSD by the promoter controlling the expression of a male-determining gene becoming constitutive instead of inducible (Valenzuela and Shikano 2007; Valenzuela 2008); males would then be determined either genetically (without the environmental trigger) or environmentally. Under this scenario, maleness would most likely be dominant (assuming dominance of mutations causing constitutive expression; Lemos *et al.* 2008), and thus females would again be the homozygous sex. Unless there is a permanent environmental change (to the female-determining environment), evolution of full GSD would require a second genetic change, abolishing the response to the environmental trigger in individuals not carrying the constitutive maleness mutation, to create females in all environments (see Figure 1). Alternatively, GSD could arise by loss-of-function mutations that abolished responses to environmental cues determining male or female development (assuming that development would then switch to the other sex). Either sex could then be the heterozygous sex. In either scenario, during the initial stages before a Y chromosome or Y-like region evolves, the factor determining maleness could recombine with the female-determining one, yielding sterile individuals, so selection would presumably favor linkage of the two genes.

Suppression of recombination and sex chromosome heteromorphism: All the organisms in Table 2 possess GSD, yet many of them lack visibly dimorphic sex chromosomes. This might suggest that, in contrast to the old and highly conserved sex chromosomes in birds, therian mammals, and Drosophila, most animal GSD systems and some of plants are quite young, and the sex chromosome pair have not yet diverged. In many animals, young homomorphic sex chromosomes may indicate a recent change in the location of the sex-determining gene (see Figure 1). However, the degree of sex chromosome heteromorphism is not a good indicator of age. First, chromosome rearrangements can produce heteromorphism, as in rainbow trout (Phillips *et al.* 2009), and even newly evolved sex chromosome systems could be heteromorphic. Furthermore, neither suppressed recombination nor heteromorphism always evolve in old-established sex chromosomes. Indeed, the homomorphic sex chromosomes in ratite birds and some reptiles clearly carry ancient GSD genes. To date, few sequences are available for comparing genes on XY or ZW pairs to estimate the time since recombination between these alleles ceased and thus a minimum age of a sex chromosome pair.

Moreover, in species with developmental plasticity in sexual differentiation, and when recombination suppression between the sex chromosomes depends on the phenotypic, rather than genotypic sex, as described above in the medaka (Kondo *et al.* 2001), occasional sex-reversed individuals may allow recombination between the Z and W or X and Y chromosomes. Old-established sex chromosomes could thus remain undifferentiated, apart from the sex-determining genes (Perrin 2009). This may be most common in poikilothermic vertebrates, where GSD is often accompanied by environmental influences, especially temperature, as in many fish, such as salmon (Devlin and Nagahama 2002) and platyfish (MacIntyre 1961). This could explain the prevalence of largely homomorphic sex chromosomes in fish (Arkhipchuk 1995). More studies of recombination between sex chromosomes in sex-reversed individuals are needed and also more studies of recombination rates in the two sexes.

The role of sexual antagonism in recombination suppression and GSD turnover: Comparative mapping in closely related species has provided new details about the diversity of GSD systems, and similar types of changes are observed in very different taxa, from plants to mammals, including fusions and translocations creating neo-sex chromosomes, gene replacements (sometimes changing the identity of the sex-determining chromosome), and transitions between male and female heterogamety. Sexual antagonism may be involved in the evolution of some of this diversity in GSD, as close linkage with a sexually antagonistic gene on an autosome can create an advantage for a new sex-determining factor (Van Doorn and Kirkpatrick 2007). Anecdotal evidence exists for turnover of sex determination due to sexual conflict in cichlid (Roberts *et al.* 2009) and stickleback fish (Kitano *et al.* 2009). The evidence for considerable sex differences in gene expression in animals (Kopp *et al.* 2003; Morrow *et al.* 2008; Mainguy *et al.* 2009; Mank 2009a) suggests that sexual antagonism is possible at many genes. However, several other models for changes in GSD exist (Bull 1983; Vuilleumier *et al.* 2007; Kozielska *et al.* 2010), and it is difficult to test which model best fits the observed patterns.

It has often been suggested that the evolution of suppressed recombination between sex chromosomes may be due to sexual antagonism. Once a sex-determining region has evolved, mutations that are advantageous in males but disadvantageous in females are particularly likely to invade a population if they arise at loci linked to the male-determining region (Fisher 1931; Rice 1984). They can then sometimes remain polymorphic, with allele frequencies differing in the two sexes, which favors reduced recombination with the sex-determining region (Bull 1983; Rice 1987; Patten and Haig 2009). Such selection evidently acts when separate sexes are newly

TABLE 2

Genetically mapped regions of sex determination in nonmodel plants and animals

Species	Type of markers	Type of GSD	Comments
Plants			
Non-flowering plants			
Ceratodon purpureus (moss)	AFLPs (McDaniel *et al.* 2007)	Males heterozygous	
Marchantia polymorpha (liverwort)	Various (Okada *et al.* 2001)	Male heterogametic	
Dicotyledons			
Rosid clade			
Vitis (grape vine, Rhamnales)	SSRs, SSCPs, morphological traits (Marguerit *et al.* 2009)	Subdioecious[a]	Three alleles for a single gene appears to confer maleness, femaleness, or dioecy
Carica papaya (papaya, Brassicales)	RAPDs, AFLPs, SSRs (Sondur *et al.* 1996; Ma *et al.* 2004; Chen *et al.* 2007a)	Male heterogametic	Male-determining Y
Basal Eudicots			
Silene latifolia (Caryophyllales)	SSCPs, genic SNP markers (Guttman and Charlesworth 1998; Bergero *et al.* 2007)	Male heterogametic	Male-determining Y, at least three genes influencing male fertility present on Y chromosome
Spinicia oleracea (spinach, Caryophyllales)	AFLPs and SSRs (Khattak *et al.* 2006)	Males heterozygous	
Rumex species (sorrel and dock, Caryophyllales)	Cytogenetics only (Bartkowiak 1971; Singh and Smith 1971)	Male heterogametic (XY or XY_1Y_2)	Male-determining Y– or X–autosome balance (Navajas-Perez *et al.* 2005)
Salix viminalis, Populus (willow, poplar and aspen, Malpighiales)	AFLPs (Semerikov *et al.* 2003); SSRs (Yin *et al.* 2008)	Females heterozygous	
Cannabis sativa (Urticales)	AFLPs (Peil *et al.* 2003)	Male heterogametic	
Humulus lupulus (hops, Rosales)	RAPD (Polley *et al.* 1997); RAPDs, AFLPs, SSRs (Seefelder *et al.* 2000)	Male heterogametic	
Fragaria virginiana (wild strawberry, Rosales)	SSRs (Spigler *et al.* (2008)	Subdioecious [a], females heterozygous	Two linked genes affecting sex determination
Asterid clade			
Actinidia chinensis (kiwifruit, Ericales)	SSRs (Fraser *et al.* (2009)	Male heterozygous	
Monocotyledons			
Asparagus officinalis (asparagus)	AFLPs (Reamon-Buttner *et al.* 1998;, Telgmann-Rauber *et al.* 2007)	Males heterozygous	Sex chromosomes may be related to Silene and Carica sex chromosomes (Telgmann-Rauber *et al.* 2007)
Dioscorea tokoro (wild yarm)	AFLPs (Terauchi and Kahl 1999)	Males heterozygous	
Animals			
Invertebrates			

(*continued*)

TABLE 2

(Continued)

Species	Type of markers	Type of GSD	Comments
Schistosoma mansoni (blood fluke)	SSRs (Criscione *et al.* 2009); FISH (Taguchi *et al.* 2007)	Female heterogametic	
Haliotis discus (Pacific abalone)	AFLPs, RAPDs, and SSRs (Liu *et al.* 2006)	Male heterogametic	
Insects			
Anopheles gambiae (mosquito)	BACs (Krzywinski *et al.* 2004)	Male heterogametic	
Culex tarsalis (mosquito)	SSRs (Venkatesan *et al.* 2009)	Males heterozygous	
Apis mellifera (honey bee)	RAPDs (Hunt and Page 1994); SSRs and SNPs (Solignac *et al.* 2004)	Haplodiploid	Complementary sex determination
Bracon (wasp)	RAPDs (Holloway *et al.* 2000)	Haplodiploid	
Bombus terrestis (buff-tailed bumblebee)	RAPDs and microsatellites (Gadau *et al.* 2001)	Haplodiploid	
Mayetiola destructor (Hessian fly)	RAPDs (Formusoh *et al.* 1996)	Male heterogametic	Postzygotic chromosome elimination (Benatti *et al.* 2009)
Bombyx mori (silkworm)	RAPDs (Abe *et al.* 1998)	Female heterogametic	W chromosome is homologous to wild ancestor, *B. mandarina.*
Allenemobius socius (ground cricket)	RAPDs and allozymes (Chu and Howard 1998)	Male heterogametic	
Leptinotarsa decemlineata (Colorado potato beetle)	AFLPs and anonymous codominant markers (Hawthorne 2001)	Male heterogametic (X0)	
Crustaceans			
Penaeus japonicus (kurua prawn)	AFLPs (Li *et al.* 2003)	Female heterogametic	
Litopenaeus vannamei (Pacific white shrimp)	AFLPs and SSRs (Zhang *et al.* 2007)	Female heterogametic	
Fish			
Catfishes (Siluriformes)			
Ictalurus punctatus (channel catfish)	SSRs (Waldbieser *et al.* 2001)	Males heterozygous	
Flatfishes (Pleuronectiforms)			
Paralichthys olivaceus (Japanese flounder)	SSRs and AFLPs (Coimbra *et al.* 2003)	Male heterozygous	
Scophthalmus maximus (turbot)	SNPs (Martinez *et al.* 2009)	Male heterozygous	
Cynoglossus semilaevis (sole)	AFLP (Chen *et al.* 2007a); BAC screening (Shao *et al.* 2010)	Female heterogametic	
Pufferfish (Tetraodontiformes)			
Takifugu rubripes (fugu)	Genetic markers (Kikuchi *et al.* 2007)	Males heterozygous	
Sticklebacks (Gasterosteiformes)			
Pungitius pungitius (ninespine stickleback)	SSRs (Shapiro *et al.* 2009); SSRs and FISH (Ross *et al.* 2009)	Male heterogametic	Sex determination is in different location from other stickleback species.
Gasterosteus aculeatus	SSRs (Peichel *et al.* 2004)	Male heterogametic	*G. wheatlandi* males are X_1X_2Y.

(*continued*)

TABLE 2

(Continued)

Species	Type of markers	Type of GSD	Comments
(threespine stickleback), *G. wheatlandi* (black-spotted stickleback)	SSRs and FISH (Ross *et al.* 2009)		
Apeltes quadracus (fourspine stickelback)	SSRs and FISH (Ross *et al.* 2009)	Females heterogametic	
Salmon (Salmoniformes)			
Salvelinus alpinus (Arctic charr), *S. namaycush* (Atlantic salmon)	SSRs and AFLPs (Woram *et al.* 2003, 2004)	Males heterozygous	Y chromosome is not orthologous to other salmonids.
Salmo salar (Atlantic salmon), *S. trutta* (brown trout)	SSRs and AFLPs (Woram *et al.* 2003); SSRs and allozymes (Gharbi *et al.* 2006)	Males heterozygous	Y chromosome is not orthologous to other salmonids.
Oncorhynchus mykiss, O. kisutch, O. clarki	SSRs and AFLPs (McClelland and Naish 2008; Woram *et al.* 2003); various (Nichols *et al.* 2003); SSRs and FISH (Alfaqih *et al.* 2008; Rexroad *et al.* 2008)	Males heterozygous	
Cichlids (Perciformes)			
Oreochromis karongae, O. aureus, O. tanganicae	SSRs and FISH (Cnaani and Kocher 2008; Cnaani *et al.* 2008)	Females heterogametic	Females heterozygous in some *O. aureus* populations (Lee *et al.* 2004)
Oreochromis niloticus (Nile tilapia)	SSRs and FISH (Cnaani *et al.* (2008)	Male heterozygous	Only in some populations (Lee *et al.* 2003)
Tilapia mariae (spotted tilapia)	SSRs and FISH (Cnaani *et al.* 2008)	Female heterogametic	
Tilapia zillii (red-bellied tilapia)	SSRs and FISH (Cnaani *et al.* 2008)	Male heterozygous	
Aulonocara baenschi, Metriaclima barlowi, M. phaeos, M. zebra, Pseudotropheus polit	SSRs (Ser *et al.* 2010)	Males heterozygous	Heterozygosity is associated with Lake Malawi cichlid linkage group 7
Labeotropheus trewavasae, Metriaclima callainos, M. fainzilberi	SSRs (Ser *et al.* 2010)	Females heterozygous	Heterozygosity is associated with Lake Malawi cichlid linkage group 5
Metriaclima pyrsonatus	SSRs (Ser *et al.* 2010)	Both male and female heterozygosity	Both Lake Malawi cichlid linkage groups 5 and 7 present in populations
Rice fish (Beloniformes)			
Oryzias latipes	Chromosome walking, FISH (Matsuda *et al.* 2002); SSRs (Kimura *et al.* 2006)	Males heterozygous	Sex determined by DMY, a recent duplicate of DMRT1
Oryzias javanicus, O. hubbsi	SNPs and FISH (Takehana *et al.* 2008)	Females heterogametic	*O. javanicus* W chromosome is not homologous to *O. hubbsi*
Oryzias luzonensis, O. dancena, O. minutillus	SNPs and FISH (Takehana *et al.* 2007; Nagai *et al.* 2008)	Males heterozygous	Y chromosomes of *O. luzonensis*, *O. dancena*, and *O. minutillus* are not homologous

(*continued*)

TABLE 2

(Continued)

Species	Type of markers	Type of GSD	Comments
Killifish and livebearers (Cyprinodontiformes)			
Nothobranchius furzeri (African killifish)	SSRs (Valenzano *et al.* 2009)	Males heterozygous	
Poecilia reticulata (guppy)	SNPs (Tripathi *et al.* 2009a)	Male heterogametic	
Xiphophorus	Allozymes and morphological markers (Morizot *et al.* 1991)	Male heterogametic	W chromosome present in some *X. maculatus* populations (Kallman 1984)
Amphibians			
Hyla arborea (European tree frog)	SSRs (Berset-Brändli *et al.* 2006)	Male heterozygous	Males are achiasmate
Ambystoma mexicanus (tiger salamander)	SNPs (Smith and Voss 2009)	Female heterozygous	
Rana rugosa (Japanese frog)	Phylogenetic inference from sex-linked gene (Miura *et al.* 1998) and FISH (Uno *et al.* 2008)	Female and male heterogametic populations	
Reptiles			
Dragon lizards (Agamidae)			
Pogona vitticeps, *P. barbata*	FISH (Ezaz *et al.* 2005) chromosome walking (Quinn *et al.* 2010)	Female heterogametic	Z and W are microchromosomes
Amphibolurus nobbi	FISH (Ezaz *et al.* 2009)	Female heterogametic	ZW sex chromosomes probably orthologous to those in *Pogona*
Ctenophorus fordi	FISH (Ezaz *et al.* 2009)	Female heterogametic	ZW sex chromosomes are not orthologous to those in *Pogona* and *Amphibolurus*

Heteromorphic sex chromosomes are termed either male or female heterogamety (XY and ZW systems, respectively), and sex-determining chromosomes without marked heteromorphism are termed male or female heterozygosity. AFLP, amplified fragment length polymorphism; BAC, bacterial artificial chromosome; FISH, florescent in situ hybridization; RFLP, random amplified length polymorphism; RAPD, random amplified length polymorphism; SNP, single nucleotide polymorphism; SSCP, single strand conformation polymorphism; SSR, simple sequence repeat, also known as microsatellites.

[a] Hermaphrodites present as well as males and females.

TABLE 3

Selective consequences that might be predicted when sex-determining regions evolve from the different potential starting states shown in Figure 1

	Possible starting states			
	Hermaphroditism (no preexisting sex-specific development)	Sex-specific developmental functions already established		
Predicted effects		Monoecy	Environmental sex determination	Preexisting GSD system
Initial genes changed	Major sterility mutations (involving at least two sex-determining genes)	Probably often major sterility mutations (involving at least two sex-determining genes)	New male or female determiners or major sterility mutations	New sex-determining gene, involving either loss- or gain- of-function mutations
Later changes in sex development and other pathways	Many other genes evolve sex-specific functions	Probably few changes	Probably few changes	Possibly several changes if sexual development is due to new genes, which interact with other pathways
Recombination suppression	Yes, initially in region with the sex-determining genes	Yes, initially in region with the sex-determining genes	Yes, initially in region with the sex-determining genes	Initially no, unless linked sexually antagonistic alleles arise in the region with the sex-determining genes

evolving and mutations at separate loci have opposite effects on male and female fertility (Charlesworth and Charlesworth 1978). After these initial mutations have become completely linked and behave as a single genetic locus, or when there is a single-locus sex-determining system because a new gene has replaced a previous system, sexually antagonistic alleles should start to accumulate at linked loci. It is currently unknown whether any new sex-determining gene that has replaced an existing one has been followed by evolution of a new nonrecombining region, but, if sexual antagonism is indeed important for evolution of nonrecombining sex chromosome regions, new sex-determining regions such as the medaka DMY region should also eventually do so. However, movement of an existing sex-determining region, creating a new sex-determining chromosome from a former autosome, may sometimes directly produce a region of suppressed recombination because of the chromosome rearrangement involved (see above).

Systems with greater levels of sexual antagonism may be the most likely to undergo progressive recombination suppression, as there are simply more sexually antagonistic loci, or loci with potentially sexually antagonistic alleles, near the sex-determining region. Highly heteromorphic sex chromosomes might therefore be most common in species with high levels of sexual conflict, and with sexual dimorphism, a likely indicator of sexual antagonism (Badyaev 2002). The male color polymorphisms in guppies and platyfish, mentioned above, are probably sexually antagonistic (Fisher 1931), as are the female coloration patterns in Lake Malawi cichlids, so these species hold promise for testing whether sexually antagonistic genes select for reduced recombination between the sex chromosomes. The scarcity of heteromorphic sex chromosomes in plants might, for instance, reflect low sexual dimorphism (Ashman 2003, 2005). However, it is not yet clear whether plant sex chromosomes evolved long enough ago for evolution of heteromorphic sex chromosomes to be expected.

Conservation in sex determination: We have emphasized changes, but it is well established that some of the downstream components of the sex determination pathway in animals are retained over long evolutionary times, even across lineages with both male- and female-heterogametic GSD, as well as ESD. DMRT1 is involved in determining maleness in many animals, ranging from invertebrates to mammals (Ferguson-Smith 2007), but it is not always on the sex chromosomes. DMRT1 maps to the Z chromosome in birds, a duplicate is located on the W chromosome in Xenopus, and the gene is important in sex determination in both clades (Yoshimoto *et al.* 2008; Smith and Voss 2009, but see Kuroiwa 2009 and Zhao *et al.* 2010), whereas, in most male heterogametic species, DMRT1 is autosomal (Ferguson-Smith 2007), and is a downstream component in sex de-

termination. The medaka fish is a notable exception where a duplicate of DMRT1 has assumed control of male development, rather as the honeybee *csd* gene has done in the very different haplodiploid sex determination system of Hymenoptera. In other cases, the earliest-acting controls have been lost from the sex determination pathway. In some mammals, the Y-linked gene SRY is the upstream regulator of DMRT1. SRY was probably recently recruited to the sex determination pathway, as it is restricted to therians (Williams and Carroll 2009), but the *SRY* gene is entirely absent in voles (Just *et al.* 1995; Chen *et al.* 2008).

In dioecious plants, very little is known about the molecular mechanisms of sex determination. Except in quite closely related plants, it is not possible to identify large regions of homologous genome, making it difficult to test whether the same chromosome carries sex-determining genes in different dioecious plants. However, it is likely that the sex-determining genes vary among taxa. Even within the genus Silene, there have been two independent origins of male heterogametic systems (Mrackova *et al.* 2008). It is estimated that large numbers of genes in plants can produce mutations with male or female sterility (Ohnishi 1985; Johnson-Brousseau and McCormick 2004; Peiffer *et al.* 2008). Different genes may therefore have undergone different sterility mutations in different dioecious taxa. One motivation for genetically mapping sex-determining regions is to identify the genes involved. In species with small sex-determining regions or single gene control, this may be possible, allowing the independent origins of sex determination to be tested, but it will remain very difficult when there is a large nonrecombining region.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

The rapid acceleration in development of genetic markers for nonmodel organisms has already made possible species- and clade-specific mapping of GSD in a variety of interesting plants and animals. Comparative genetic mapping of GSD in closely related clades can reveal whether apparently similar types of GSD are indeed due to shared ancestry and have the same sex-determining region, or whether changes have occurred in the location, and perhaps the identity, of the genes controlling sex determination. This can provide information about the directions of the changes that have occurred, a necessary preliminary to asking about the selective mechanisms of changes in sex determination and about how elements of the sex determination pathway can evolve while conserving the important functions.

Even without identifying the actual genes involved in sex determination, genetic linkage mapping of GSD in more taxa will reveal nonrecombining regions in species where recombination suppression has evolved. Linkage mapping can reveal X–Y and Z–W orthologs, which can then be used to date the divergence of the sex chromosomes and aid in understanding the age of GSD systems. This type of information on more and more diverse taxa will be needed to understand the evolutionary patterns and time course of events involved in the evolution of GSD and of sex chromosomes. Without such information, it will not be possible to understand the selective forces underlying these changes.

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